THE PRESENT STATUS OF THE ETIOLOGY OF PRIMARY ATYPICAL PNEUMONIA*

COMMISSION ON ACUTE RESPIRATORY DISEASES**

JOHN H. DINGLE, *Director*

PRIMARY atypical pneumonia has been well recognized as a distinct clinical entity during the past six years.[1-3] Except in a small proportion of cases, the syndrome may be differentiated readily from the common bacterial pneumonias. Epidemiological observations have been made of the disease under conditions of endemic and epidemic spread.[4-7] The possible relationship of atypical pneumonia and undifferentiated acute respiratory disease has been pointed out by several investigators.[3-7]

Although the clinical and epidemiological aspects of primary atypical pneumonia have been quite well characterized, much uncertainty and obscurity still exist with respect to the cause of this syndrome. It has been recognized for some time that similar clinical illnesses may be produced by certain well-known agents. The relatively minor role of such agents in the production of atypical pneumonia as seen today, however, has not been emphasized.

We wish to present tonight a review of the present status of the etiology of primary atypical pneumonia and a summary of the studies carried out by the Commission on Acute Respiratory Diseases in an attempt to transmit this disease to animals and to human volunteers.

ATYPICAL PNEUMONIA CAUSED BY KNOWN AGENTS

The clinical syndrome of atypical pneumonia may be produced in

* This investigation was supported through the Commission on Acute Respiratory Diseases, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, United States Army, and by grants from the Commonwealth Fund, the W. K. Kellogg Foundation, the John and Mary R. Markle Foundation, and the International Health Division of the Rockefeller Foundation to the Board for the Investigation and Control of Influenza and Other Epidemic Diseases for the Commission on Acute Respiratory Diseases.

** Presented October 16, 1944 by John H. Dingle, Major, M.C., Director, before the Seventeenth Graduate Fortnight of The New York Academy of Medicine. From the Respiratory Diseases Commission Laboratory, Regional Hospital, Section 2, Fort Bragg, North Carolina.
Members and professional associates of the Commission on Acute Respiratory Diseases are: John H. Dingle. Major, M.C., A.U.S., Director; Theodore J. Abernethy, Major, M.C., A.U.S.; George F. Badger, Captain, M.C., A.U.S.; Joseph W. Beard, M.D.; Norman L. Cressy, Major, M.C., A.U.S.; A. E. Feller, M.D.; Irving Gordon, M.D.; Alexander D. Langmuir, Captain, M.C., A.U.S.; Charles H. Rammelkamp, Jr., M.D.; Elias Strauss, Captain, M.C., A.U.S.; and Hugh Tatlock, 1st Lieutenant, M.C.. A.U.S.

its essential characteristics by a variety of agents, including bacteria, fungi, Rickettsia, and viruses.[1-3] An etiological diagnosis can be established in these cases by the use of procedures such as intradermal tests, isolation of the agent, and immunological or serological tests with the patients' acute-phase and convalescent-phase sera. When positive results are obtained, the illness must be considered as a pneumonia of specific etiological type and the diagnosis of primary atypical pneumonia should not be employed. In this respect, particular mention should be made of the psittacosis-lymphogranuloma or ornithosis group of viruses. Several recent publications have indicated that these viruses cause many cases of so-called primary atypical pneumonia.[8-17] While there is no doubt that localized outbreaks as well as sporadic cases of pneumonia due to these agents can occur, the data now accumulating indicate that the great majority of cases of primary atypical pneumonia are not caused by the psittacosis or related viruses.[6, 12, 18] During the past three years of the Commission's experience in the Army, the diagnosis of psittacosis or ornithosis has not once been established in a series of more than 500 patients with atypical pneumonia and other respiratory infections.

The bacteria, fungi, Rickettsia, and viruses known to produce pneumonia must therefore be sought and excluded as a cause of illness in any study of primary atypical pneumonia. It now appears to be established, however, that all of these known agents taken together account for an exceedingly small proportion of the total number of cases diagnosed as primary atypical pneumonia, in civilian as well as in military populations.

Primary Atypical Pneumonia of Unknown Cause

Extensive investigations have been carried out during the past few years in a number of laboratories in an attempt to transmit primary atypical pneumonia of undetermined cause. In most of the work laboratory animals of various species have been employed. Isolation of the causative agent has been claimed or implied in several recent publications.

Animal experimentation. In 1939, Stokes, Kenney, and Shaw[19] reported the transmission of a pneumotropic agent from the sputa of patients with atypical pneumonia to ferrets and mice. Unfortunately the agent was lost after several passages in mice and its serological relationship to the patients' illnesses was not established. The following

year Weir and Horsfall[20] reported the recovery from patients with acute pneumonitis of a virus causing pneumonia in the mongoose. Experiments with other animal species yielded negative results. The specimens employed were obtained from patients whose illnesses were consistent with a diagnosis of primary atypical pneumonia. The agent could apparently be maintained in chick embryos without the production of lesions. On transmission in the mongoose, lesions were produced with some irregularity. Neutralization with convalescent sera of the patients could not be obtained on initial inoculation of serum-virus mixtures, but was evidenced from a lower percentage of lesions in the mongooses by serial passages, in contrast to the results when acute-phase serum-virus mixtures were employed.

It may be of significance that the above two investigations were carried out on cases occurring during a period when atypical pneumonia attracted the attention of physicians in several Eastern cities.[21-23] While it is likely that recognition alone accounted for the reported prevalence of the disease, it is also possible that the cases were unique etiologically.

In the past three years several other reports have appeared. Blake, Howard, and Tatlock[24] isolated a pneumotropic agent from cats ill concomitantly with human cases of atypical pneumonia in a single household. Neutralization tests, though incomplete, suggested that the human and feline illnesses might have been due to the same agent. Baker[11] likewise isolated an agent from feline pneumonia and suggested its causal relationship to human atypical pneumonia. Baker's agent, however, subsequently proved to be a virus of the psittacosis-lymphogranuloma group.[15,25,26]

By the intranasal inoculation of cotton rats with sputa from cases of atypical pneumonia in the Army, Johnson[6] obtained pulmonary lesions. On passage of the lungs of these animals, however, lesions failed to persist with any degree of constancy.

Eaton, Meiklejohn, Van Herick, and Talbot[27,28] also found lesions in cotton rats and hamsters inoculated with sputa and lungs from patients with atypical pneumonia. More consistent results were obtained by the inoculation of chick embryos with material from the patients and subsequent inoculation of cotton rats and hamsters with the chick embryo tissue. Using this technique, it was found that convalescent sera from patients with atypical pneumonia prevented the production of lesions by the chick embryo tissue in most of the animals.

Horsfall and his coworkers[29] likewise obtained lesions by direct intranasal inoculation of cotton rats with sputa from certain cases of atypical pneumonia, but the lesions were not maintained on passage. These investigators further found that many of the animals, if permitted to survive, developed neutralizing antibodies for the pneumonia virus of mice.[30] Convalescent sera from patients with atypical pneumonia, when mixed with the sputum prior to inoculation, prevented the development of such antibodies, although these sera themselves contained no antibodies to the mouse virus. It was subsequently found[31] that antibodies to the pneumonia virus of mice could be stimulated non-specifically in cotton rats by inoculation of substances such as broth. Thus the appearance of these antibodies may well result from evoking a latent virus, but the ability of convalescent atypical pneumonia sera to prevent the development of the antibodies remains an unexplained observation.

Rose and Molloy[32] reported the occurrence of pulmonary lesions in newly-weaned guinea pigs inoculated intranasally with specimens from patients ill with atypical pneumonia. Immunological confirmation of the relation of such lesions to atypical pneumonia could not be obtained however, and Rose[33] now holds the opinion that no causal association exists.

Sanders[34] has isolated a virus in tissue culture and mice from the sputum of a case of atypical pneumonia. The virus produces a fatal encephalitis in mice but fails to cause pulmonary lesions. A strain of this virus, kindly supplied by Major Sanders, has been studied in the Commission laboratory. The development of antibodies to this agent during the course of atypical pneumonia could not be demonstrated by the neutralization test in mice. The agent appears to be a variant of the herpes virus.

An unusual combination of immunological reactions can be demonstrated with convalescent sera from cases of atypical pneumonia. They are, first, cold hemagglutination; second, fixation of complement with a variety of dissimilar antigens; third, prevention of the development of antibodies to the mouse pneumonia virus; and last, agglutination of an indifferent streptococcus. In general, the reactions are found with greatest constancy in sera from the most severely ill patients.

The development of cold agglutinins for group O human erythrocytes in the sera of patients with primary atypical pneumonia has been reported by a number of investigators.[35-40] The proportion of cases

showing this reaction has varied in different series from about 30 per cent to almost 100 per cent, depending in all probability on the factors of selection which entered into the availability of the cases. In an Army hospital, for example, a large number of patients with atypical pneumonia have such mild illnesses that they would not be hospitalized in civilian life. Thus far, no clues to the causative agent of atypical pneumonia have been afforded and substantiated by the reaction of cold hemagglutination.

Thomas and his co-workers[41] have reported that the sera of certain patients convalescing from primary atypical pneumonia have the capacity to fix complement with a variety of antigens, particularly those consisting of fresh suspensions of tissue. Fixation occurred with suspensions of various organs from different animal species, regardless of whether the animals were normal or infected with one of several viruses. These results have been confirmed on a limited scale in the Commission laboratory.

The capacity of convalescent sera of cases of atypical pneumonia to prevent the development of antibodies to the pneumonia virus of mice in animals inoculated with sputum[29] has already been discussed.

In 1943, Thomas and his co-workers[42, 43] at the Rockefeller Institute reported the isolation of an indifferent streptococcus from lungs and sputa of certain cases of primary atypical pneumonia. This organism is agglutinated by convalescent-phase sera from some patients with atypical pneumonia. It is closely related immunologically to *Streptococcus salivarius*, type I[44] and owes its serological reactivity primarily to a capsular polysaccharide. The organism in pure culture is not pathogenic for experimental animals. Cross-adsorption tests have shown that the agglutinins for the streptococcus are distinct from cold agglutinins for human group O erythrocytes. At the present time, the role of the bacterium in the causation of primary atypical pneumonia is obscure.

The convalescent sera of many cases of primary atypical pneumonia thus have the capacity to react immunologically in a variety of ways and with grossly dissimilar antigenic substances. While it is possible that these substances all have antigens in common with the causative agent of atypical pneumonia, yet another tenable hypothesis is that the various reactions reflect an alteration in the serum proteins and their reactivity, which is demonstrable non-specifically.

Repeated attempts to detect or isolate the causal agent or agents of

primary atypical pneumonia have been made in the Commission laboratories during the past three years. Throat washings, sputa, and blood from more than sixty cases of atypical pneumonia have been utilized. In addition, lung, spleen, brain, and other tissues from six fatal cases have been studied. In so far as possible, bacteria, fungi, Rickettsia and known viruses were sought for and excluded as causative agents in these cases.

Throat washings, sputa and lung have been subjected to fractional ultracentrifugation. The various fractions so obtained have been used in animal experiments as inocula, and in complement-fixation tests as antigens with acute-phase and convalescent-phase sera from cases of atypical pneumonia. The results failed to indicate the presence of any specific agent. Examination of the fractions with the electron microscope revealed no characteristic particles not also found in control specimens.

Extensive animal experimentation has been done, employing various routes and methods of inoculation, with and without adjuvants, such as mucin. No agents which could be related definitely to the human disease have been isolated in chick embryos, chickens, doves, Java ricebirds, mice, guinea pigs, ferrets, rabbits, dogs and three species of monkeys. Similar negative results were obtained with cotton rats and kittens. A series of experiments[45] employing the mongoose as the experimental animal has been carried out in the Antilles Department Medical Laboratory in Puerto Rico with the collaboration of Major G. J. Dammin and Captain T. H. Weller. Throat washings from seven cases of primary atypical pneumonia, sputa from six cases, lung from two fatal cases, and control specimens were inoculated intranasally and passed serially in a total of 265 mongooses. An agent was not isolated, nor could clinical or pathological evidence of pulmonary infection be demonstrated in these animals.

A great deal of the animal experimentation in the Commission Laboratory has been difficult to interpret due to the occurrence of nonspecific or spontaneous pulmonary lesions following intranasal inoculation and passage. At times, latent agents which could be identified have been evoked, such as the pneumonia virus of mice and viruses of the psittacosis-lymphogranuloma group. At other times, lesions have appeared in one passage, only to disappear completely on subsequent passage of those lungs. Extensive controls have been employed and in no instances have lesions appeared following inoculation of material from

patients with atypical pneumonia, which could not be duplicated in control series.

Thus, the occurrence of non-specific lesions and possibly the evocation of latent agents may account for much of the confusion and conflicting reports in the literature regarding the causative agent of primary atypical pneumonia. At all events, it would appear that detailed control series must be employed at all stages in animal experimentation—not only for attempting isolation of an agent, but also for relating the agent so obtained to the human disease.

Human experimentation: In view of the equivocal results in animal investigations, it seemed desirable to study experimentally the transmission of primary atypical pneumonia in the natural host, man. One such study has been reported. Vance, Scott, and Mason[46] were unsuccessful in transmitting the disease to seven volunteers by intranasal inoculation with filtrates of sputa and nasal washings.

The experiments summarized below have been carried out in human beings to determine (a) whether or not the disease is transmissible with secretions of the respiratory tract of ill patients, and (b) whether or not bacteria-free filtrates of such secretions are capable of inducing infection. The subjects were conscientious objectors who volunteered after the nature of the investigation had been completely explained to them.

The first experiment[47] was conducted in October, 1943, in a Civilian Public Service camp near Gatlinburg, Tennessee. A roentgenographic survey of the entire personnel of the camp failed to reveal any cases of atypical pneumonia. Accordingly, fifteen volunteers were placed in group isolation in the camp's infirmary. During the next five days, the men were examined daily for evidence of respiratory disease. Three individuals developed mild, afebrile upper respiratory infections during this time and were not inoculated. None of the three developed evidence of pulmonary involvement at this period or subsequently. Despite this complication, the remaining twelve men were inoculated with a pool of untreated sputa and throat washings from seven cases of atypical pneumonia. The sputa and throat washings were mixed and sprayed from an atomizer and nebulizer into the nose and pharynx of each volunteer, synchronizing the spraying with his inhalations.

Respiratory illnesses developed in ten of the twelve volunteers inoculated. These illnesses varied in their clinical characteristics, severity, and course. In two of the men the infections were mild, without fever.

In the remaining eight cases, fever developed from approximately 7 to 22 days after the inoculations. Three of the eight showed no evidence of pneumonia at any time, but characteristic "sticky," subcrepitant rales were present in the lungs of 5 patients. The roentgenograms of three of these patients showed minimal patchy infiltration in one or both lower lobes, and their illnesses were consistent clinically with moderately severe atypical pneumonia. The illnesses of the other two patients were similar to those considered as "suspected atypical pneumonia" or "bronchitis resembling atypical pneumonia," in that the clinical illness and physical signs were characteristic of atypical pneumonia, but pulmonary infiltration was not demonstrable roentgenographically. The sera of three of the men showed cold agglutinins in titers of 64, 128, and 256, respectively, during the course of their illness. None of the patients developed agglutinins for the indifferent streptococcus or antibodies for the influenza viruses.

These preliminary results were encouraging, even though interpretation was complicated, first, by the occurrence of mild respiratory infections in three of the group before the time of inoculation, secondly, by the possibility of cross-infection among the twelve inoculated men, and, lastly, by the failure of any of them to develop pronounced pulmonary infiltration. None the less, the illnesses of three of the men, following inoculation, were clinically characteristic of atypical pneumonia, and no similar illnesses occurred in the other personnel of the camp during the period of the experiment. Moreover, the infections were neither similar to the common cold clinically,[48] nor to influenza clinically and immunologically.

The second experiment was carried out in June and July, 1944. Thirty-six volunteers were placed in isolation in individual rooms equipped with private baths on the 2nd and 3rd floors of a 75-room hotel. Quarantine was instituted to prevent chance exposure to atypical pneumonia through outside contact, and was maintained for a period of three weeks prior to inoculation, since the available evidence suggested this interval as the maximal limit of the incubation period. The usual isolation techniques of a contagious hospital were instituted and maintained. Complete examinations, including roentgenographic, electrocardiographic and laboratory studies were done. The men were observed on alternate days for symptoms and signs of respiratory infection. Any one with acute or chronic organic or infectious disease was rejected.

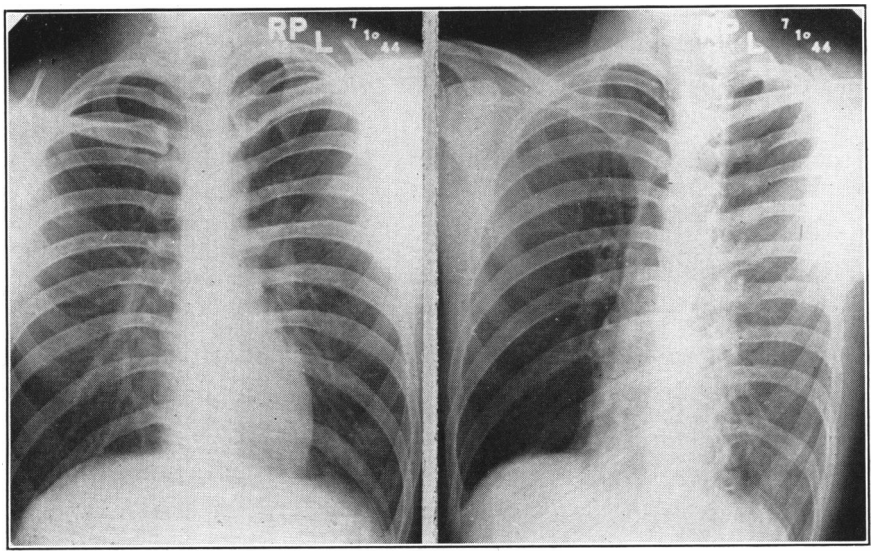
After three weeks of isolation and observation, the men were inoculated in three groups of twelve persons each, as follows: (a) untreated throat washings and sputa pooled from seven cases of primary atypical pneumonia, (b) the same material, filtered through sintered glass and Seitz filters, and (c) the same material, autoclaved. The inoculations were made on a single day, utilizing first floor rooms located in one wing of the hotel as remote from the rooms of the volunteers as was possible. Three separate rooms, on the same side of the hall, were employed for the three types of inocula. Sheets, wet with cresol solution, were hung over the doors outside the rooms. The doors were opened frequently to admit entrance or exit of the volunteers and investigators.

Each man received a total inoculum of 10 ml. administered in three equally divided doses by spraying in synchronization with deep inhalation. The material was sprayed by three methods: hand atomizer, hand nebulizer, and an atomizer powered by a motor-driven air pump —the power spray commonly employed by otolaryngologists. The power spray was used first for the filtered material, secondly for the autoclaved material, and finally for the untreated inoculum. The equipment was sterilized between each set of inoculations, with the exception of the inner surface of the air pump on the power spray which was overlooked. The volunteers and professional personnel were masked and gowned in an attempt to prevent cross-infection or extraneous exposure. Precautions were taken to insure consistency and comparability in the inoculations of the three groups of men.

Ten cases of atypical pneumonia developed among the thirty-six volunteers. The cases varied in severity but all were characteristic of the disease as it has occurred in the Army. The following charts and brief case summaries will illustrate the essential character of the clinical illnesses and radiographic findings.

Cold hemagglutinin titers of 64 or greater developed in the sera of seven of the ten cases of atypical pneumonia during the course of illness. Agglutinins for the indifferent streptococcus, Rockefeller strain No. 344, developed during convalescence in the sera of two cases in a maximum titer of 32 from a pre-inoculation titer of <8. No change in titer was detected in the other eight cases.

Repeated attempts were made to isolate the indifferent streptococcus by culture from the volunteers both before and after inoculation. The organism was found in one-third of the men before inoculation and in



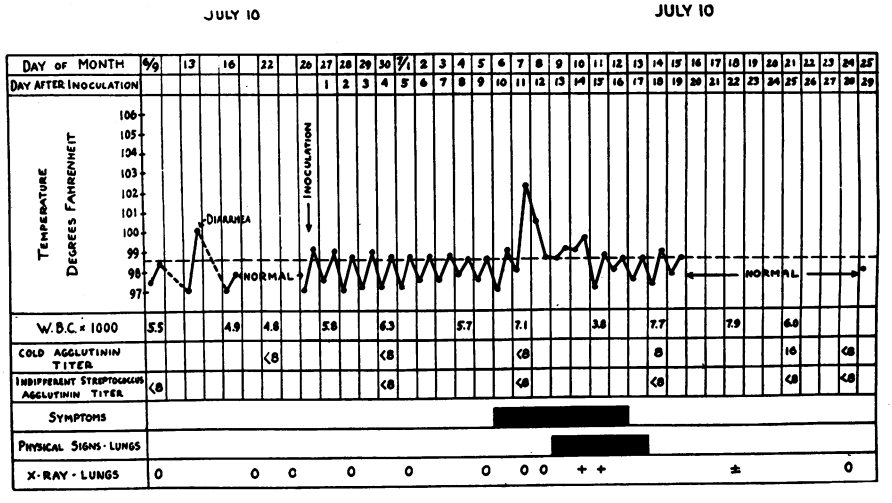
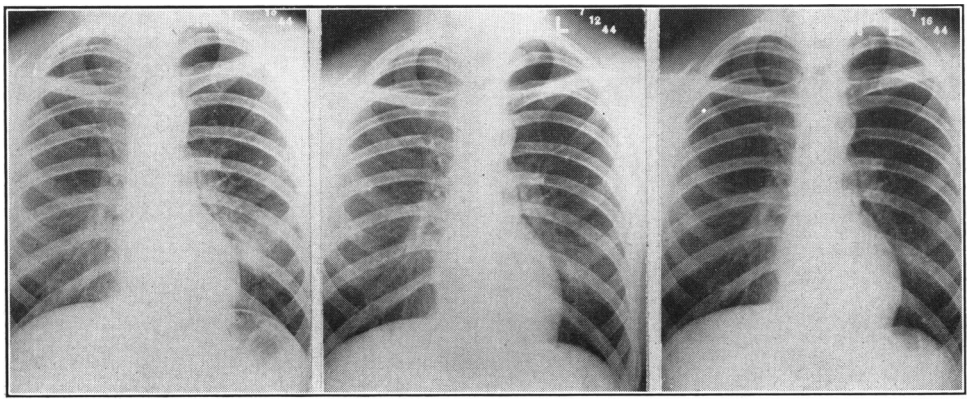


Figure 1—A mild case of primary atypical pneumonia. This patient experienced a mild episode of gastro-enteritis during the period of quarantine. Recovery was prompt and complete. On the tenth day after inoculation he developed mild atypical pneumonia, demonstrable by roentgenogram, in the left lower lung field. Cold hemagglutinins and agglutinins for an indifferent streptococcus (#344) did not develop. (Case No. 46)



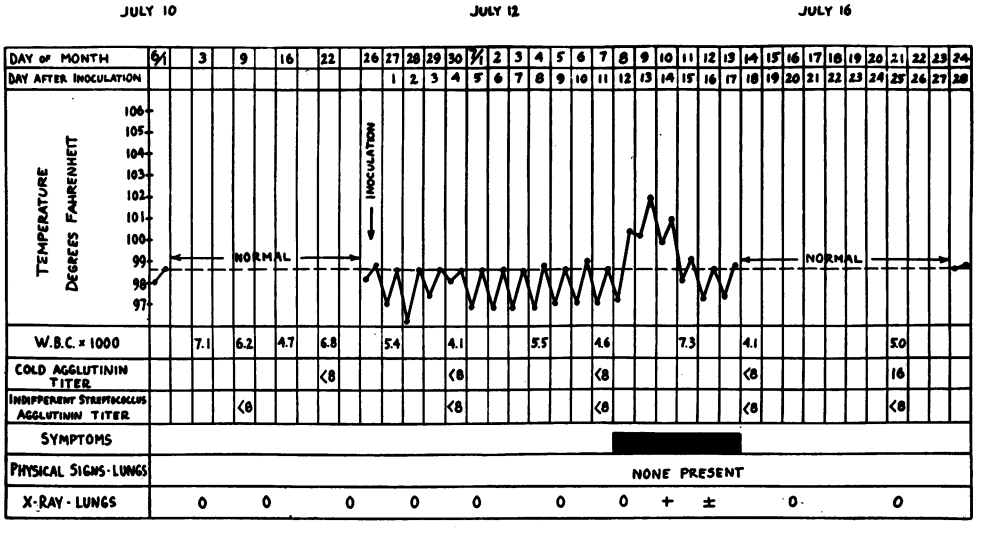
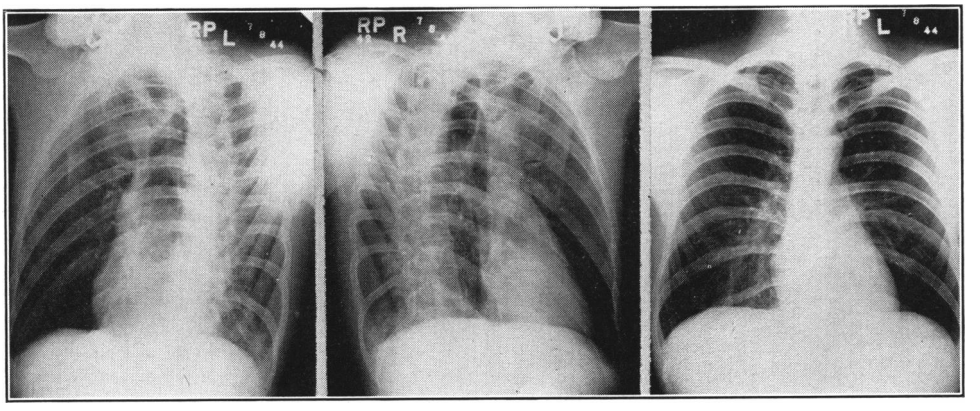


Figure 2—(Case No. 25). A mild case of primary atypical pneumonia. Mild atypical pneumonia began abruptly in this patient 12 days after inoculation. Roentgenogram on the 14th day (July 10th) showed a soft infiltration in the left lower lobe. No abnormal physical signs in the lungs were detected at any time. Neither cold hemagglutinins nor agglutinins for streptococcus #344 were detected in his convalescent sera.



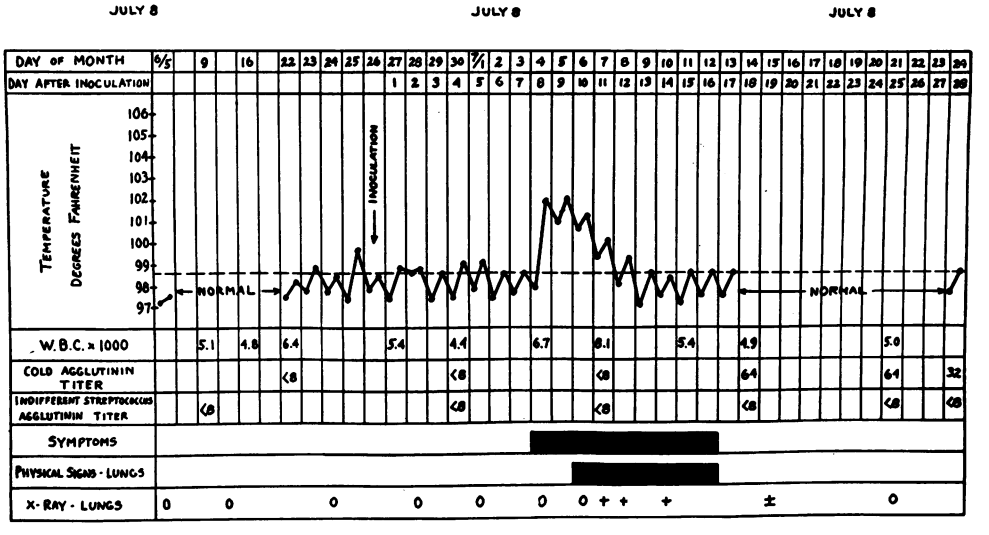
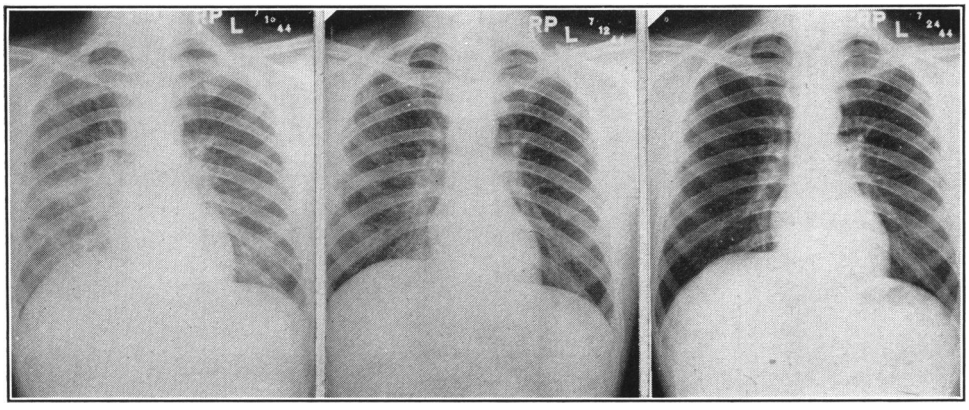


Figure 3—(Case No. 42). A mild case of primary atypical pneumonia with pulmonary infiltration visualized roentgenographically only in the right and left oblique positions. This patient experienced a mild attack of atypical pneumonia beginning on the 8th day after inoculation. Physical signs of pulmonary involvement were present in both bases. Areas of infiltration at the extreme lung bases were visualized roentgenographically only in the right and left oblique positions and were not visible in the conventional posterior-anterior films. Convalescent sera showed a maximum cold hemagglutinin titer of 64; agglutinins for the indifferent streptococcus were not found.



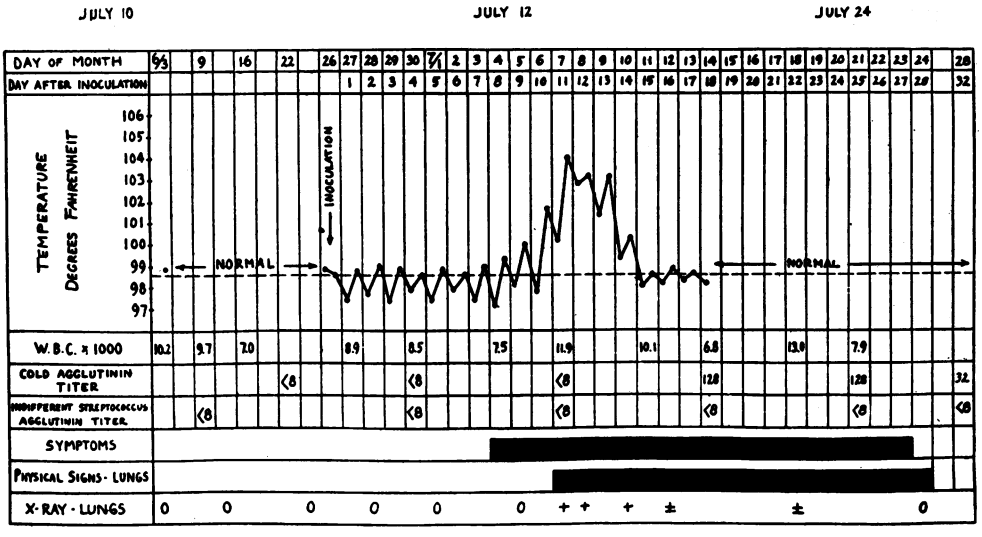
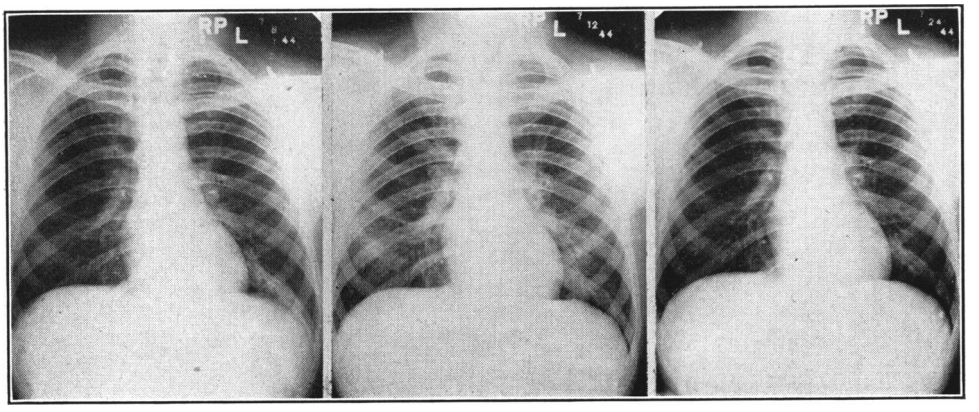


Figure 4—(Case No. 2). A moderately severe case of primary atypical pneumonia with involvement of the entire right lung and left lower lobe. Moderately severe atypical pneumonia developed in this patient 8 days after inoculation. The infiltration ultimately involved the entire right lung and the left lower lobe. Cold hemagglutinins developed to a titer of 128. No agglutinins for the indifferent streptococcus were demonstrable.



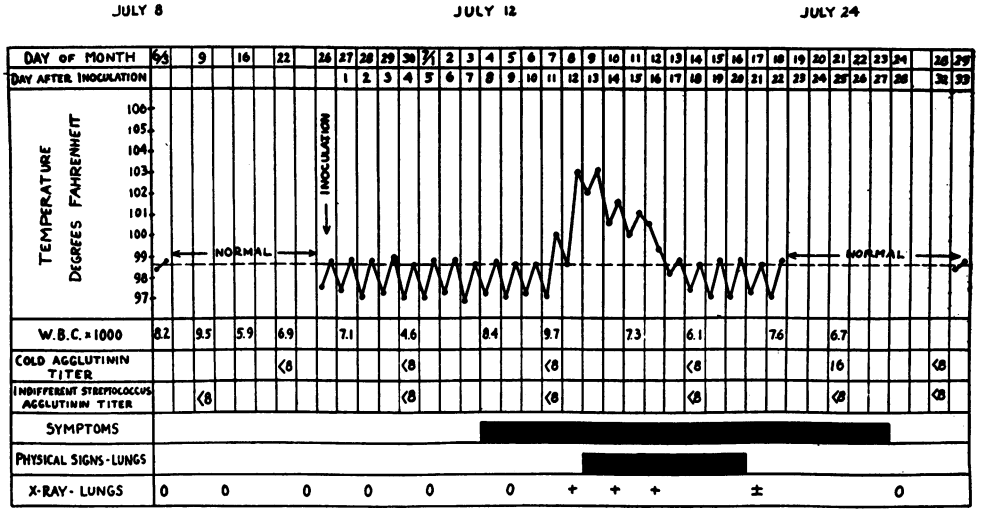
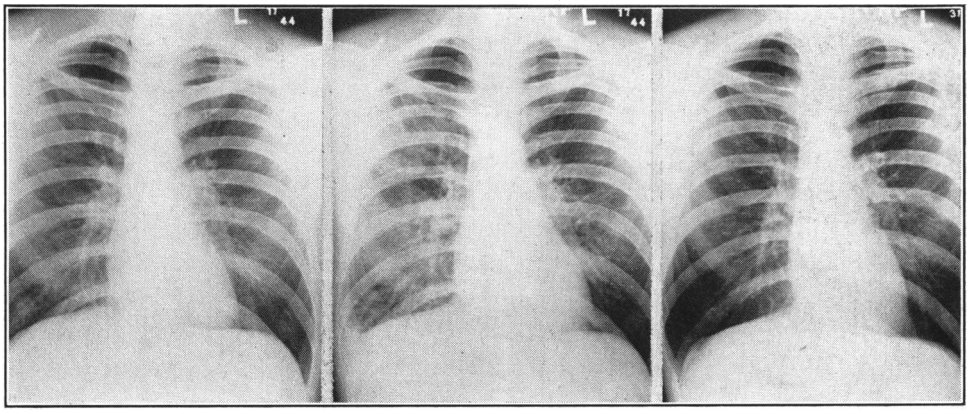


Figure 5—(Case No. 5). A moderately severe case of primary atypical pneumonia. This patient experienced moderately severe atypical pneumonia. Symptoms appeared 8 days after inoculation and the temperature became elevated 3 days later. Pneumonic infiltration appeared first in the left lower lobe and subsequently involved the right lower lobe. Abnormal physical signs were present at both bases. Neither cold hemagglutinins nor agglutinins for streptococcus #344 were found in the convalescent sera in significant titers.



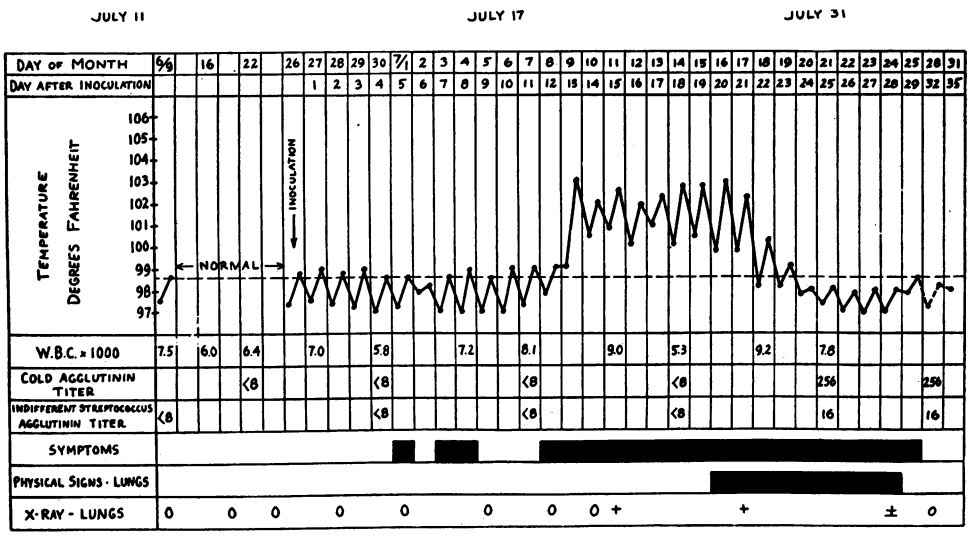
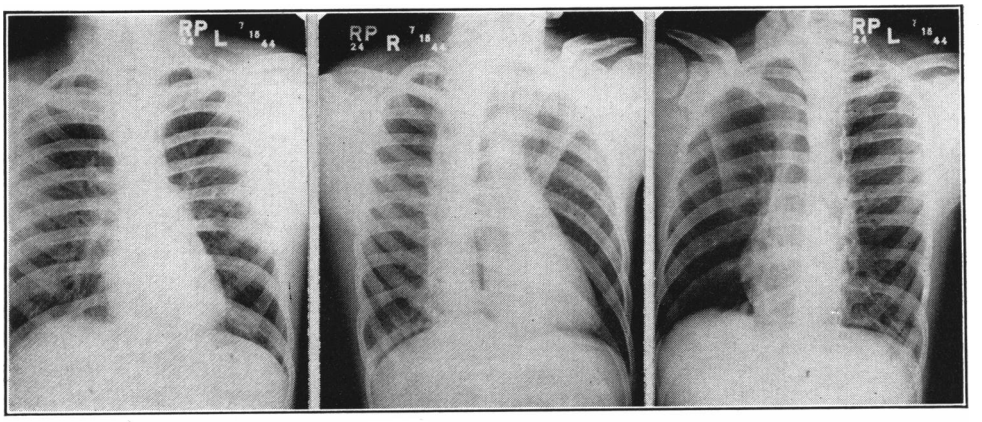


Figure 6—(Case No. 45). A moderately severe case of primary atypical pneumonia. After 2 intermittent periods of respiratory symptoms, this patient became ill with moderately severe atypical pneumonia on the 12th day, post-inoculation. Remittent fever persisted for 10 days. Patchy pneumonic infiltration of the entire right lung and left lower lobe were demonstrable in roentgenograms. Cold hemagglutinins were present in a maximum titer of 256 on the 25th day after inoculation, or 13 days after onset of illness. Streptococcus agglutinins increased to a titer of 16 at the same time and reached a titer of 32, 4 weeks later.



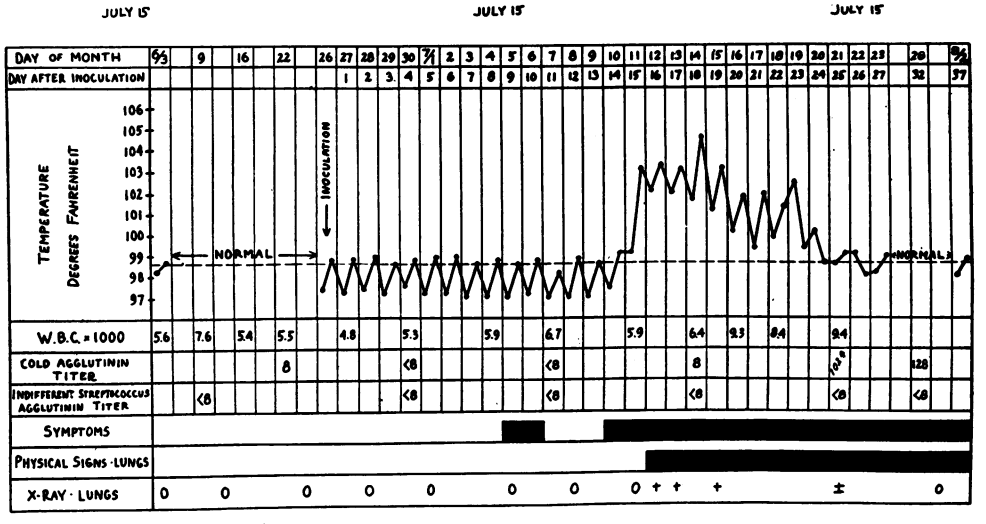
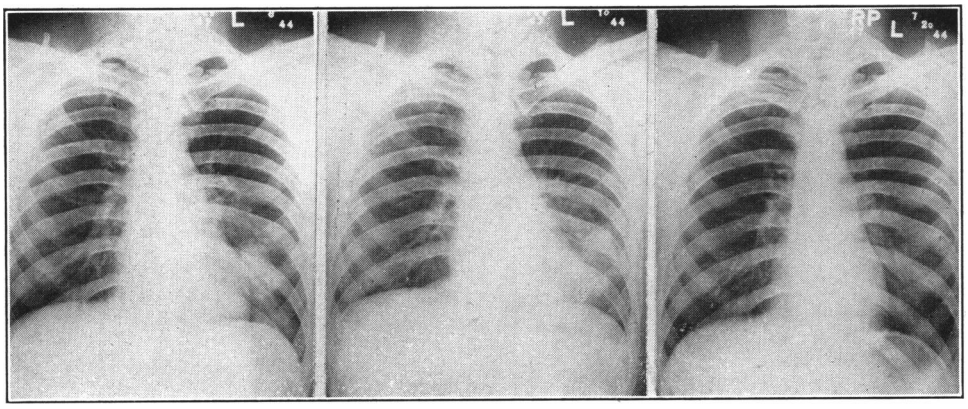


Figure 7—(Case No. 24). A severe case of primary atypical pneumonia complicated by disorientation and emotional instability. This patient's illness was characterized by high temperature and patchy infiltration at both lung bases on July 15th, the 19th day after inoculation. He was considerably sicker than the extent of his lesions would suggest. Following the peak of temperature of 104.6° F., disorientation and emotional instability were conspicuous features of his illness. Neurological examination revealed no abnormal findings, however, and the cerebrospinal fluid was normal. Recovery was complete. Cold hemagglutinins were demonstrable in a titer of 1024 on the 11th day after onset of illness. Agglutinins for streptococcus #344 were not found.



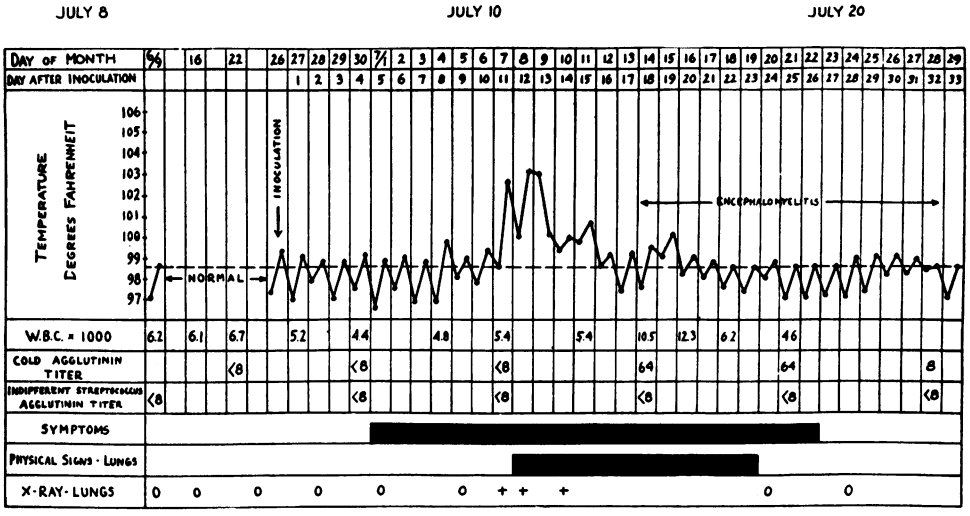
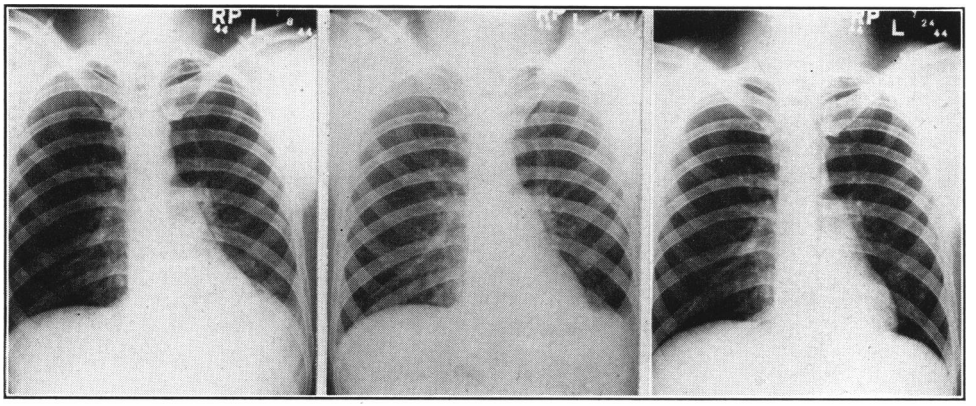


Figure 8—(Case No. 47). A relatively mild case of primary atypical pneumonia complicated by myeloencephalitis. This patient suffered from a relatively mild atypical pneumonia with involvement of the left lower lobe. On the 18th post-inoculation day, however, symptoms and signs of meningeal irritation appeared. Neurological findings indicative of diffuse myeloencephalitis developed during the succeeding days. Cerebrospinal fluid was clear and contained 17 small mononuclear cells per cubic millimeter. Chemical determinations were within normal limits and the fluid was sterile. Recovery was gradual but complete. The titer of cold hemagglutinins reached a maximum of 64; agglutinins for the indifferent streptococcus were not detected.



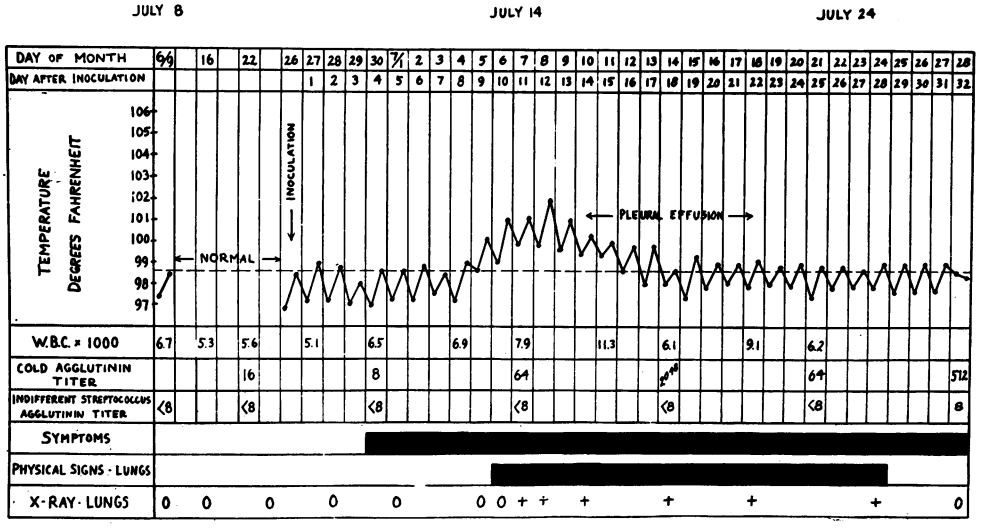
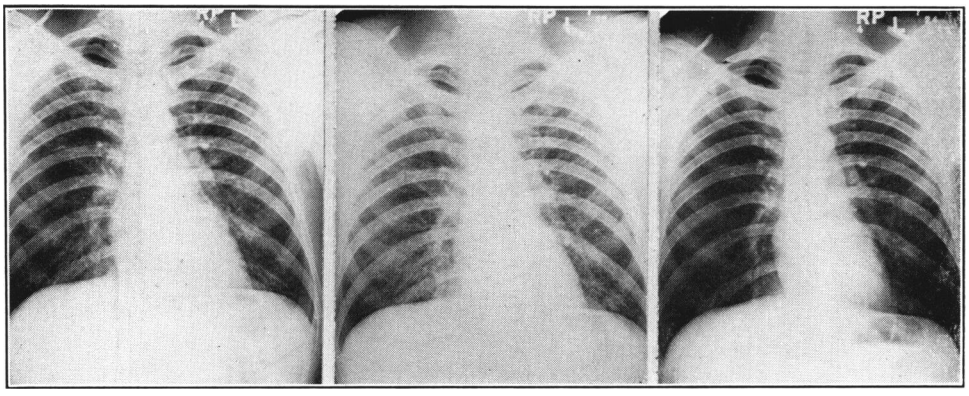


Figure 9—(Case No. 44). A relatively mild case of primary atypical pneumonia complicated by sterile pleural effusion. In this volunteer, atypical pneumonia, though relatively mild in character, was complicated by the development of pleural effusion at the left base. The fluid was sterile, greenish-yellow in color, and contained 1370 leukocytes and 240 erythrocytes per cubic millimeter. Approximately 55 per cent of the leukocytes were large mononuclear cells; the remainder were lymphocytes and neutrophiles. Cold hemagglutinins were present in the patient's serum in a maximum titer of 2048. Agglutinins for streptococcus #344 were not present in significant titer.



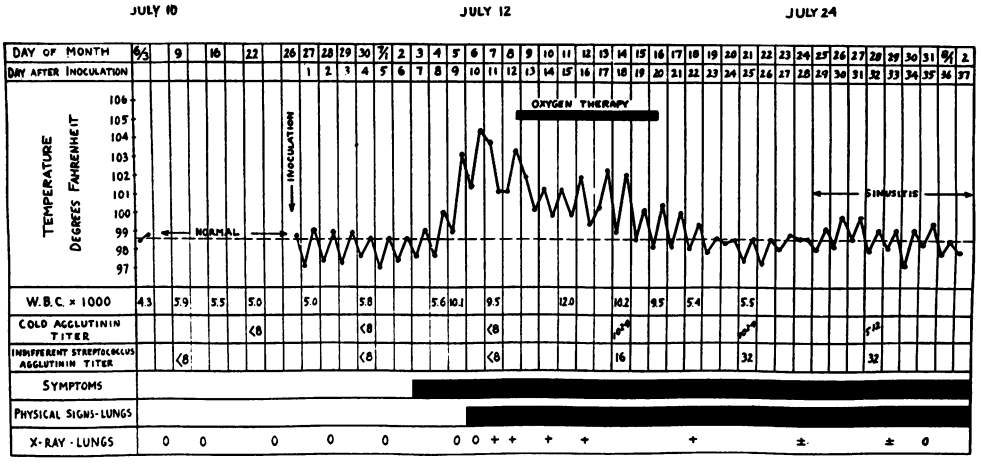


Figure 10—(Case No. 4). A severe case of primary atypical pneumonia with involvement of all lobes of the lung. This case represents the most severe atypical pneumonia in the group. Pulmonary infiltration appeared first in the right upper and middle lobes and subsequently extended to involve all five lobes. By roentgenogram a fine diffuse mottling was present throughout the lung fields. Oxygen therapy was required for approximately a week. Sinusitis occurred as a complication. Recovery was gradual but ultimately complete. Cold hemagglutinins and streptococcus agglutinins reached titers of 1024 and 32, respectively, in the convalescent sera.

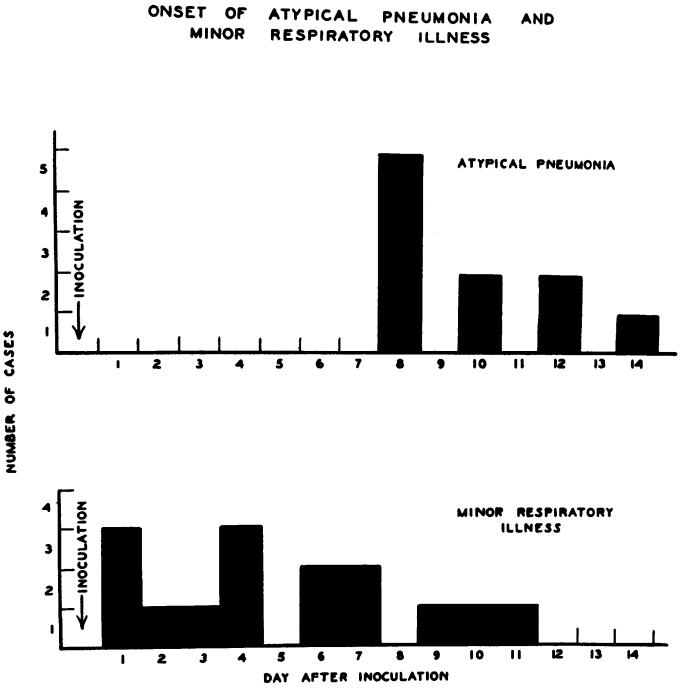


Figure 11.

one-half of them after inoculation, but the increase occurred with equal frequency in those who developed atypical pneumonia and in those who did not. There was, therefore, no apparent relation between the presence of this organism, either before or after inoculation, and the development of atypical pneumonia.

In addition to the ten cases of primary atypical pneumonia, there were fifteen cases of minor respiratory illness of undifferentiated type. The clinical onset of these cases, as well as that of the cases of atypical pneumonia, in relation to inoculation is shown in figure 11. For the purpose of this chart, the clinical onset of atypical pneumonia was defined as the day of development of *persistent* symptoms or signs which resulted in atypical pneumonia. The clinical onset of minor respiratory illness was defined as the day of development of any recognizable symptom or sign which was a definite departure from the normal state for that individual but did not lead to atypical pneumonia. This distinction was necessary because many of the minor respiratory illnesses were of short duration.

The clinical onset of atypical pneumonia, as thus defined, varied

TABLE I

PRODUCTION OF PRIMARY ATYPICAL PNEUMONIA AND MINOR RESPIRATORY ILLNESS IN HUMAN VOLUNTEERS INOCULATED WITH UNTREATED, FILTERED, AND AUTOCLAVED SPUTA AND THROAT WASHINGS FROM HOSPITALIZED CASES

Inoculum	*No. Men*	*RESULT*		
		AP	*MRI*	*No Illness*
Untreated	12	3	9	0
Filtered	12	4	5	3
Autoclaved	12	3	1	8
Total	36	10	15	11

AP—Primary atypical pneumonia.

MRI—Minor respiratory illness of undifferentiated type.

from 8 to 14 days after inoculation, whereas the onset of minor respiratory illness varied from one to eleven days. If the criteria used for minor respiratory illness were applied to the cases of atypical pneumonia, however, the onset of illness was between one and twelve days after inoculation. Whether or not these results indicate the presence of multiple agents capable of causing respiratory illness or merely mild illnesses or prodromata due to a single agent, cannot be determined at present. In none of the cases was there any change in bacterial flora, after inoculation or during illness, to suggest causation or even secondary bacterial infection. No serological evidence was obtained indicating infection with a virus of the psittacosis-lymphogranuloma group.

The results of this experiment clearly indicated that primary atypical pneumonia, and in addition, minor undifferentiated respiratory illness could be transmitted with secretions of the respiratory tract of patients with atypical pneumonia. Further analysis of the results, however, revealed that atypical pneumonia was apparently produced without regard to the type of inoculum (Table I), that is, three cases occurred with untreated inoculum, four with filtered inoculum, and three with the autoclaved material. The production of minor respiratory illness indicated greater specificity in that nine cases occurred with untreated

inoculum, five with filtered material, and only one with autoclaved inoculum. The interval between inoculation and onset of illness was in general shortest in the group receiving untreated sputa and throat washings.

A detailed review of all the conditions and procedures of the experiment was then undertaken in the hope of finding an explanation for the results. Numerous possibilities were considered but the three most likely explanations appeared to be (a) air-borne infection in the hallway on the day of inoculation, (b) contamination of the inner surface of the air pump for the power spray, and (c) the non-specific evocation of a latent agent. In support of air-borne infection was the occurrence of atypical pneumonia in an attendant who assisted in inoculating twelve volunteers with the untreated inoculum. Contamination of the air pump of the power spray could have been at least a contributing factor. During its first use with filtered inoculum, the air taken into the pump undoubtedly contained air-borne particles which were deposited on the inner surface of the pump. These particles could again have been liberated when the power spray was used with the autoclaved material. It is perhaps significant in this respect that, of the twelve men receiving autoclaved material, the three who developed atypical pneumonia were the first, second, and fourth in order of inoculation with the power spray. The possibility of non-specific evocation of a latent agent could only be determined by repetition of the experiment using extreme precautions against air-borne or other types of inadvertent "cross-infection."

The third experiment was then begun in August, 1944 as a repetition of the second, but with the following differences:

a. The inoculum consisted of pooled sputa and throat washings from 6 cases of atypical pneumonia in the second experiment, thus providing an opportunity for passage of the agent.
b. The inoculations were performed out-of-doors.
c. A tank of nitrogen was used as a source of pressure in place of the motor-driven power spray.
d. An interval of four days was allowed to elapse between inoculation of the autoclaved material and the filtered material. A second interval of 8 days elapsed before administration of the untreated inoculum.
e. Eighteen men were included in the group which received auto-

TABLE II

PRODUCTION OF PRIMARY ATYPICAL PNEUMONIA AND MINOR RESPIRATORY ILLNESS IN HUMAN VOLUNTEERS INOCULATED WITH UNTREATED, FILTERED, AND AUTOCLAVED SPUTA AND THROAT WASHINGS FROM EXPERIMENTALLY PRODUCED CASES OF ATYPICAL PNEUMONIA

Inoculum	*No. Men*	*RESULT*		
		AP	*MRI*	*No Illness*
Untreated	12	3	5	4
Filtered	12	3	5	4
Autoclaved	18	0	1	17
Total	42	6	11	25

AP—Primary atypical pneumonia.

MRI—Minor respiratory illness of undifferentiated type.

claved material to increase the possibility of demonstrating the evocation of a latent agent, if such were the cause.

f. The groups of men were segregated geographically within the building by construction of temporary partitions in the hallways.

In other respects, the essential conditions of the experiment were the same.

Six cases of atypical pneumonia and eleven cases of minor respiratory infection occurred in this experiment. Three of the cases of minor respiratory infection were diagnosed as "suspected atypical pneumonia" or "bronchitis resembling atypical pneumonia," because of the characteristic clinical course and physical signs, without roentgenographic evidence of pulmonary infiltration. In general, the illnesses were somewhat less severe than those in the preceding experiment, but in other respects they were entirely characteristic.

Distribution of the cases with respect to type of inoculum is shown in Table II. The untreated material produced three cases of atypical pneumonia and five cases of minor respiratory illness of which two were instances of "suspected atypical pneumonia." In the group of men receiving filtered material, there were likewise three cases of atypical pneumonia and five cases of minor respiratory illness, but only one of

the latter could be diagnosed as "suspected atypical pneumonia." There were no cases of atypical pneumonia among the eighteen men inoculated with autoclaved material, and only one instance of minor illness. This case occurred in the only man, to our knowledge, who broke isolation. On at least one occasion, he descended the fire escape to the room below which was occupied by a volunteer who subsequently developed a minor respiratory illness diagnosed as "suspected atypical pneumonia."

A striking difference was noted in the incubation period of the illnesses. The clinical onset of illnesses in the men receiving untreated material, with one exception (14 days), occurred between five and eight days after inoculation, whereas it was between nine and fifteen days, or almost twice as long, with the filtered inoculum.

The results of this experiment thus demonstrate that primary atypical pneumonia may be produced in human beings with filtered as well as untreated secretions of the respiratory tract of patients ill with this disease.

Discussion and Summary

An attempt has been made to review the present status of the etiology of primary atypical pneumonia. While a small proportion of cases presenting this clinical syndrome are due to known bacteria, fungi, or viruses, the cause of the majority of them remains to be characterized and identified.

The results of animal experimentation are confusing and difficult to interpret, due in large part to the lack of a truly susceptible animal, as well as to the complications introduced by the occurrence of spontaneous diseases causing pulmonary lesions in the animals employed. No work has yet been reported, and confirmed by other investigators in the field, which describes the isolation in animals of an agent clearly related immunologically to the human disease.

The experiments in human volunteers, summarized here, demonstrate that respiratory disease has been induced by the administration of sputa and throat washings of patients with primary atypical pneumonia. The clinical types of disease varied from the mildest of undifferentiated respiratory illness to classical severe atypical pneumonia. Between these extremes were cases resembling atypical pneumonia in onset, course, and physical findings, but lacking roentgenographic con-

firmation of pulmonary infiltration. Such cases were entirely similar to those observed on the respiratory wards of Army hospitals and diagnosed as "bronchitis resembling atypical pneumonia" or "suspected atypical pneumonia." The varied types of respiratory disease produced in these volunteers thus are consistent with the hypothesis previously advanced on the basis of clinical and epidemiological observations,[3-7] namely, that primary atypical pneumonia may be a severe form with pulmonary involvement of the same infection responsible for a large part of the more common, mild respiratory illnesses.

From these experiments some information may be gained regarding human susceptibility to respiratory diseases. Excluding the controls inoculated with autoclaved material, three-fourths of the men developed some type of respiratory illness and one-fourth had atypical pneumonia. In spite of the relatively large dose of 10 ml., one-fourth of the men had no illness whatsoever. Direct comparison of these figures, however, with attack rates observed in civilian and military populations is probably not justified because of lack of knowledge regarding minimal infecting dosage.

The question of the transmission of primary atypical pneumonia with bacteria-free filtrates of sputa and throat washings from patients with this disease was not easily answered. The results of the second experiment, in which atypical pneumonia was apparently produced with autoclaved inocula, permitted no conclusion regarding infectivity of the filtrates. They furthermore raised the question of non-specific evocation of a latent agent as the cause of the disease, as well as the possibility of inadvertent infection.

Accordingly, the third experiment was carried out utilizing extreme precautions against inadvertent infection and increasing to 18 the number of men in the control group, to strengthen the likelihood of detecting non-specific evocation. Sputa and throat washings from the previous experimental cases were employed as inocula to establish serial passage. The results demonstrated that primary atypical pneumonia could be induced with bacteria-free filtrates of sputa and throat washings as well as with the same inoculum before filtration. Autoclaved material, however, was without effect. It therefore appeared probable that the agent was filterable, that a latent agent was not evoked non-specifically, and that the cases following inoculation of autoclaved material in the prior experiment resulted from inadvertent infection. More-

over, from experimentally produced cases of atypical pneumonia, this illness as well as undifferentiated respiratory disease could again be transmitted to well individuals.

The length of time between inoculation and onset of illness may be of some significance, since this interval was almost twice as long in the cases receiving filtered inoculum as in those inoculated with untreated material. It is possible that such an increase in the incubation period is a function of dosage, and that filtration caused a considerable loss of the infecting agent. Another explanation is that bacteria act in conjunction with a filterable agent in the production of this disease. The failure to detect changes in bacterial flora following inoculation and during illness does not support, nor does it completely exclude, this concept.

The study in human volunteers thus leads to the conclusion that primary atypical pneumonia is at least initiated, if not caused, by a filter-passing agent, presumably a virus.*

* ACKNOWLEDGMENT—The members of the Commission wish to express their gratitude to the men who volunteered as subjects and attendants in the experiments reported here, and to the administrative staffs of Selective Service, the National Service Board for Religious Objectors and the American Friends Service Committee for the coöperation which made the studies possible. The Commission is indebted to Brigadier General H. C. Coburn, Jr., U.S.A., Colonel J. C. Williams, M.C., Colonel R. C. Tatum, M.C., Lieutenant Colonel F. J. Pohle, M.C., and Lieutenant Colonel F. K. Herpel, M.C., for their coöperation and assistance; to 1st Lieutenant Clara B. Kwash, A.N.C., 2nd Lieutenant Catherine E. Hammond, A.N.C. and Mrs. Catherine Kruse who served as nurses; and to Sergeant A. P. Aan, X-ray technician.

The Commission takes pleasure in acknowledging the assistance of the technical staff of the laboratory: Pfc. Alexander Adler, T/5 David O. Foltz, Corp. Melvin H. Kaplan, T/5 Gerald J. Leuty, Barbara A. Mulliken, 2nd Lieutenant Walter A. Mickle, Sn.C., 1st Lieutenant Thomas J. Oliver, Sn.C., 1st Lieutenant Ralph L. Robinson, Sn.C., and Irene A. Salamandra.

REFERENCES

1. Finland, M. and Dingle, J. H. Medical progress; virus pneumonias; pneumonias associated with known non-bacterial agents; influenza, psittacosis and Q fever, *New England J. Med.*, 1942, *227*:342.
2. Dingle, J. H. and Finland, M. Medical progress; virus pneumonias; primary atypical pneumonias of unknown etiology, *New England J. Med.*, 1942, *227*: 378.
3. MacLeod, C. M. Primary atypical pneumonia, *M. Clin. North America*, 1943, *27*:670.
4. Reimann, H. A. and Havens, W. P. An epidemic disease of the respiratory tract, *Arch. Int. Med.*, 1940, *65*:138.
5. Iverson, H. A. Epidemic of acute respiratory disease associated with atypical pneumonia, *Bull. Johns Hopkins Hosp.*, 1943, *72*:89.
6. Dingle, J. H. *et al.* Primary atypical pneumonia, etiology unknown, *Am. J. Hyg.*, 1944, *39*:67; 197; 269.
7. Commission on Acute Respiratory Diseases. Epidemiology of atypical pneumonia and acute respiratory disease at Fort Bragg, North Carolina, *Am. J. Pub. Health*, 1944, *34*:335.
8. Eaton, M. D., Beck, M. D. and Pearson, H. E. Virus from cases of atypical pneumonia; relation to viruses of meningopneumonitis and psittacosis, *J. Exper. Med.*, 1941, *73*:641.

9. Meyer, K. F., Eddie, B. and Yanamura, H. Y. Ornithosis (psittacosis) in pigeons and its relation to human pneumonitis, *Proc. Soc. Exper. Biol. & Med.*, 1942, *49*:609.
10. Eaton, M. D. and Corey, M. Complement-fixation in human pneumonitis with group-reactive virus antigens, *Proc. Soc. Exper. Biol. & Med.*, 1942, *51*:165.
11. Baker, J. A. Virus obtained from pneumonia of cats and its possible relation to cause of atypical pneumonia in man, *Science*, 1942, *96*:475.
12. Smadel, J. E. Atypical pneumonia and psittacosis, *J. Clin. Investigation*, 1943, *22*:57.
13. Favour, C. B. Ornithosis (psittacosis); report of 3 cases and historical, clinical and laboratory comparison with human atypical (virus) pneumonia, *Am. J. M. Sc.*, 1943, *205*:162.
14. Beck, M. D., Eaton, M. D. and O'Donnell, R. Further laboratory studies on classification of psittacosis-like agents, *J. Exper. Med.*, 1944, *79*:65.
15. Baker, J. A. Virus causing pneumonia in cats and producing elementary bodies, *J. Exper. Med.*, 1944, *79*:159.
16. Furth, J. and deGara, P. F. Granular body characteristic of certain non-bacterial pneumonias of mice, *Proc. Soc. Exper. Biol. & Med.*, 1944, *56*:107.
17. Meiklejohn, G. B., Beck, M. D. and Eaton, M. D. Atypical pneumonia caused by psittacosis-like viruses, *J. Clin. Investigation*, 1944, *23*:167.
18. Commission on Acute Respiratory Diseases. *Unpublished data.*
19. Stokes, J., Jr., Kenney, A. S. and Shaw, D. R. New filtrable agent associated with respiratory infections, *Tr. & Stud. Coll. Physicians, Philadelphia*, 1938-39, *6*:329.
20. Weir, J. M. and Horsfall, F. L., Jr. Recovery from patients with acute pneumonitis of virus causing pneumonia in the mongoose, *J. Exper. Med.*, 1940, *72*:595.
21. Reimann, H. A. Acute infection of the respiratory tract with atypical pneumonia; disease entity probably caused by a filtrable virus. *J.A.M.A.*, 1938, *111*:2377.
22. Kneeland, Y., Jr. and Smetana, H. F. Current bronchopneumonia of unusual character and undetermined etiology, *Bull. Johns Hopkins Hosp.*, 1940, *67*:229.
23. Longcope, W. T. Bronchopneumonia of unknown etiology (variety X); report of 32 cases with 2 deaths, *Bull. Johns Hopkins Hosp.*, 1940, *67*:268.
24. Blake, F. G., Howard, M. E. and Tatlock, H. Feline virus pneumonia and its possible relation to some cases of primary atypical pneumonia in man, *Yale J. Biol. & Med.*, 1942, *15*:139.
25. Thomas, L. and Kolb, E. M. Relationship of the virus of cat pneumonia (Baker) to the psittacosis-lymphogranuloma group of agents, *Proc. Soc. Exper. Biol. & Med.*, 1943, *54*:172.
26. Rake, G. and Jones, H. P. Association of specific toxins with agents of the lymphogranuloma-psittacosis group, *J. Exper. Med.*, 1944, *79*:463.
27. Eaton, M. D., Meiklejohn, G., Van Herick, W. and Talbot, J. C. Infectious agent from cases of atypical pneumonia apparently transmissible to cotton rats, *Science*, 1942, *96*:518.
28. Eaton, M. D., Meiklejohn, G. and Van Herick, W. Filterable agent transmissible to cotton rats, hamsters, and chick embryos, *J. Exper. Med.*, 1944, *79*:649.
29. Horsfall, F. L., Jr. *et al.* Virus recovered from patients with primary atypical pneumonia, *Science*, 1943, *97*: 289.
30. Horsfall, F. L., Jr. and Hahn, R. G. Latent virus in normal mice capable of producing pneumonia in its natural host, *J. Exper. Med.*, 1940, *71*:391.
31. Curnen, E. C. Symposium on primary atypical pneumonia, *Soc. Am. Bacteriologists, General Meeting*, May 1944.
32. Rose, H. M. and Molloy, E. Observations concerning the etiology of primary atypical pneumonia, *Science*, 1943, *98*: 112.
33. Rose, H. M. Symposium on primary atypical pneumonia, *Soc. Am. Bacteriologists, General Meeting*, May 1944.

34. Sanders, M. *Personal communication.*
35. Clough, M. C. and Richter, I. M. Study of an autoagglutinin occurring in a human serum, *Bull. Johns Hopkins Hosp.*, 1918, *29*:86.
36. Peterson, O. L., Ham, T. H. and Finland, M. Cold agglutinins (autohemagglutinins) in primary atypical pneumonias, *Science,* 1943, *97*:167.
37. Turner, J. C. Development of cold agglutinins in atypical pneumonia, *Nature,* 1943, *151*:419.
38. Horstmann, D. M. and Tatlock, H. Cold agglutinins; diagnostic aid in certain types of primary atypical pneumonia, *J. A. M. A.,* 1943, *122*:369.
39. Meiklejohn, G. Cold agglutination test in the diagnosis of primary atypical pneumonia, *Proc. Soc. Exper. Biol. & Med.,* 1943, *54*:181.
40. Commission on Acute Respiratory Diseases. Cold hemagglutinins in primary atypical pneumonia and other respiratory infections, *Am. J. M. Sc.,* 1944, *208*:742.
41. Thomas, L. *et al.* Complement fixation with dissimilar antigens in primary atypical pneumonia, *Proc. Soc. Exper. Biol. & Med.,* 1943, *52*:121.
42. Thomas, L. *et al.* Serological reactions with an indifferent streptococcus in primary atypical pneumonia, *Science,* 1943, *98*:566.
43. Horsfall, F. L., Jr. and Thomas, L. *Personal communication.*
44. Sherman, J. M., Niven, C. F., Jr., and Smiley, K. L. Streptococcus salivarius and other non-hemolytic streptococci of the human throat, *J. Bact.,* 1943, *45:* 249.
45. Commission on Acute Respiratory Diseases in collaboration with Dammin, G. J. and Waller, T. H. Attempt to transmit primary atypical pneumonia and other respiratory tract infections to the mongoose, *J. Immunol.,* 1945, *50*:107.
46. Vance, D. H., Scott, T. and Mason, H. C. Inability to pass primary atypical pneumonia to human volunteers, *Science,* 1943, *98*:412.
47. Commission on Acute Respiratory Diseases. An experimental attempt to transmit primary atypical pneumonia in human volunteers, *J. Clin. Investigation, in press.*
48. Dochez, A. R., Shibley, G. S., Mills, K. C. Studies in the common cold; experimental transmission of common cold to anthropoid apes and human beings by means of a filtrable agent, *J. Exper. Med.,* 1930, *52*:701.